UNIVERSITAS INDONESIA

The Effectiveness of *Bacillus thuringiensis israelensis* in Controlling *Aedes aegypti* Larvae in Water Containers in Rawasari Village, Central Jakarta

RESEARCH REPORT

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FACULTY OF MEDICINE
INTERNATIONAL CLASS PROGRAM
JAKARTA
JULY 2013

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RESEARCH REPORT

A final project report presented to Universitas Indonesia
in partial fulfilment of the requirements for the
Degree of *Sarjana Kedokteran* (Bachelor of Medicine)

Dwi Permana Putra

0806471840

FACULTY OF MEDICINE
INTERNATIONAL CLASS PROGRAM
JAKARTA
JULY,2013

STATEMENT OF ORIGINALITY

This final project (undergraduate) is of my own composition. Any and all outside resources, whether quoted or referenced, have been stated as such

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ACKNOWLEDGMENT

The author sends praises and thankfulness to Allah SWT for the guidances and blessings all the way through the progress of this final assignment. The author would like to express his honest gratitude to the following people whom had shared with him their understanding and experience. Without their supervision, support, and helpful suggestion, there would not be an abundant knowledge gained at the end of this project.

Dr.dr. Ratna Sitompul,Sp.M the dean of FKUI, and Dr. Dewi Irawati, coordinator of International Class FKUI for giving precious supports since the writer starts to conduct the project. Prof. Saleha Sungkar, for many valuable suggestion and support so that the author can finished his final project. Her patience and compassion mean a lot for the author. And also Dr. Retno Asti, who taught the author about the right way for processing research data and transform it into a final report. For fellow group-mates Seno Triadi, Rheza Maulana, Intan Mutia, and Ivana Firman, for their continuous support since the research group was established. Finally, the author hopes that this project would contribute for the development of Parasitological science, especially in the terms of *Aedes aegypti* controls.

Jakarta,8 July 2013
The Author

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ABSTRAK

Nama : Dwi Permana Putra
Program Studi : Pendidikan Dokter
Judul : Efektivitas dari *Bacillus thuringiensis israelensis* dalam memberantas larva *Ae.aegypti* pada container (TPA) di Kelurahan Rawasari, Jakarta Pusat

Demam Berdarah Dengue (DBD) merupakan penyakit yang menjadi masalah kesehatan masyarakat di DKI Jakarta,salah satunya di Kelurahan Rawasari, Jakarta Pusat. Untuk memberantas vektor DBD salah satu cara yang dapat dilakukan adalah menggunakan biolarvasida, yaitu *Bacillus thuringiensis israelensis* (Bti). Tujuan penelitian ini adalah untuk mengetahui efektivitas dari larvasida Bti dalam pemberantasan vektor DBD di Container TPA. Desain penelitian ini adalah menggunakan metode *quasi*-eksperimental dengan intervensi aplikasi Bti bentuk larutan konsentrasi 4 ml/m^2. Data sebelum intervensi diambil pada tanggal 14 Februari 2010 dan sesudah intervensi pada tanggal 15 Maret 2010 yang terletak Kelurahan Rawasari, Jakara Pusat. Survei entomologi dilakukan dengan *single-larval method* di container TPA di 100 rumah. Data diolah dengan program SPSS versi 20 dengan analisis menggunakan uji McNemar. Hasil menunjukkan bahwa setelah pemberian Bti terjadi penurunan dari 15 container positif menjadi 12 container, tetapi, hasil McNemar menunjukkan p = 0,629, artinya tingkat kepadatan larva *Ae.aegypti* tetap tinggi. Maka, Bti konsentrasi 4 ml/m^2 tidak efektif dalam menurunkan keberadaan larva *Ae.aegypti* di container TPA.

Kata kunci : *Ae.aegypti*, Pemberantasan *Bacillus thuringiensis israelensis*, Rawasari, TPA

ABSTRACT

Name : Dwi Permana Putra
Study Program : Bachelor of Medical Science
Title : The Effectiveness of *Bacillus thuringiensis israelensis* in Controlling *Ae.aegypti* Larvae in Water Containers in Rawasari Village, Central Jakarta

Dengue Haemorrhagic Fever (DHF) is a disease that has become a public health problem in Jakarta, including Rawasari Village. One of the way that can be done to control the dengue vector is using biolarvacide, that is known as *Bacillus thuringiensis israelensis* (Bti). The purpose of this study is to examine the effectiveness of Bti larvacide in controlling dengue vectors in water containers. This research is using *quasi-experimental* design with intervention of Bti applications liquid form with 4 ml/m^2 of concentration. Before Bti application which was taken on 14 of February 2010 and after Bti applications on 15 of March 2010 in Rawasari Village, Jakara Pusat. The entomology survey is conducted with *single-larval container* method in water containers of 100 houses. The data is processed using SPSS version 20 with and analyzed by using McNemar test. Results showed that after application of Bti, the number of *Ae.aegypti* larvae decreased from 15 to 12 of positive larvae. As a result the McNemar test shows P = 0.629, which means that the density of *Ae.aegypti* larvae remains high. So that, Bti cannot reduce the existence of *Ae.aegypti* larvae in the water containers with the concentration of 4 ml/m^2.

Keywords : *Ae.aegypti*, Larvae, *Bacillus thuringiensis israelensis*, water container, Rawasari Village

TABLE OF CONTENTS

PICTURE LISTS

LIST OF TABLES

PART 1
INTRODUCTION

Dengue haemorrhagic fever (DHF) is a disease that caused by the dengue virus and transmitted by the *Ae.aegypti* mosquito as the main vector and *Ae.albopictus* as secondary vectors. Common symptoms which are appears in the DHF patients such as headache, fever, severe joint, exhaustion, muscle pain, swollen glands (lymphadenopathy), and rash in the development of DHF). There are three things that involved in the transmission of DHF, namely the dengue virus, *Ae.aegypti* mosquitoes and human as *host.* Moreover, there are three factors that are influenced of the transmission of DHF, including biologic environmental, physical environment and immunity by host. [1-2]

DHF disease remains as a public health problem in Indonesia, because the number of DHF patients continue to rise and spread widely, especially in the big cities. The highest number of DHF patients was reported in DKI Jakarta.[3] In 2004, the number of DHF patients has reached 20.640 people and 90 of them died. In 2005 the number of DHF patients has reached 23.466 people and 80 of them died, while in 2006 DHF patients increased to 24.932 people and 51 of them died. Furthermore, in 2007 the number of DHF patients rose to 31.836 people and 87 of them died. In 2008, the number of DHF patients decreased to 28.327 people and 26 people died. However, in January and February 2009 there were found about 4.920 of DHF patients. One of the endemic area of DHF is in Jakarta Pusat. In this region, there are nine villages are known as red zone, which means these areas that over three weeks, there are more than nine patients suffered by DHF disease or there are some people who die due to DHF disease.[4]

Rawasari Village is reported as the third highest areas that found many of DHF cases in Jakarta Pusat. Although this area is small, the number of DHF patients in 2009 has reached 108 cases and relatively high in 2010 which accounted for 152 cases.[5] The controlling of adult mosquitoes, carried out by spraying (fogging) with the organosphosphate insecticide, synthetic pyrethroids and carbamates. The controlling of *Ae.aegypti* larvae are usually known as a control of mosquito breeding (PSN), Chemical (i.e larvacide temephos), biology (nurture larvae-eating fish, such as fish heads and fish guppy tin) and physical commonly known as 3M (*menguras, menutup, mengubur*). For example, cleaning the "bak mandi" and drum, also bury or destroy cans or tyres used). However, the depletion of containers need to be done regularly at least once a week, thus mosquitoes can not breed in these places. But, because of people are busy and many of them do not have a maid and water price is expensive so that they do not to do 3M.

Meanwhile, fogging cannot be done continuously is because it is expensive, also causing pollution to the environment and causing resistance. Therefore, other agents are needed to control dengue vectors that are cheap and do not have negative impacts to environment or people. One of these agents that can be used is *Bacillus thuringiensis israelensis* which is gram-positive bacteria forming a spore and proteins that is insecticide called δ-endotoksin.[6-8] One strain of *Bacillus thuringiensis israelensis* (Bti) has long been used to eradicate *Anopheles* because it is effective to use it at a low dose and safe for humans and the environment. Currently, Bti has been formulated to be used for controlling *Ae.aegypti* larvae and has been used in many countries including in Cuba. In Indonesia, an examination of Bti has been done in the laboratory, but the effectiveness and how to control the distribution of *Ae.aegypti* larvae in the field is still unknown. Bti is available in small bottle with the concentration for 2-5 ml/m^2. To the environments with clear water conditions, low concentrations of Bti can be used which is only 2 ml/m^2, while in the dirty environments used it in higher concentrations. Increased the concentrations of BTI can be done based on arithmetically or geometrically. For dangerous microbes used to arithmetically and not harmless microbes that use geometrical progression.[10]

Because of *Ae.aegypti* often breeding in the clear water, thus in this study the students have to use Bti with the concentrations of 2 ml/m^2 to control *Ae.aegypti* in the Rawasari Village. The breeding places for *Ae.aegypti* larvae is in the containers that filled with clear water or slightly polluted water such as a "bak mandi", drum, water tank, cans used, vases, and place the bird drinks. Containers can be divided into water containers (TPA) and non-water containers (non-TPA). Therefore, this study needs to be done in the water containers and non-water containers, but in this assignment is only discuss about using Bti in the water containers.

1.2 Research questions:

• How does the effect of *Ae.aegypti* larvae existences in the water containers before and after application 4 ml/m of Bti in Rawasari Village?

1.3 Hypothesis

The existence of *Ae.aegypti* larvae would decrease due to application 4 ml/m^2 of Bti

1.4 Objectives

1.4.1 General Objectives

To know about the existences of *Ae.aegypti* larvae in Rawasari Village before and after Bti applications.

1.4.Specific Objective

1.4.2.1 To know about the presence of *Ae.aegypti* larvae before applications of Bti
1.4.2.2 To know about the presence of *Ae.aegypti* larvae after applications of Bti
1.4.2.3 To know about the proportion of the existence of *Ae.aegypti* larvae before and after Bti applications

1.5. Benefits

1.5.1. Benefits for researchers:

1. Self-training interaction and communication between peers
2. Applying the medical knowledge gained from studying at Faculty of Medicine University of Indonesia
3. Facilitates and studying of conducting research in the biomedical aspects

4. Improving the instincts, point of view, critical thinking, creativity, analytical and systematical reasonings, and interest in the research for identification of the public health problems
5. Gaining basic knowledge and experiences of conducting the research
6. Train the group teamwork.

1.5.2. Benefits for Institutions:

1. To applying the "Tri Darma Perguruan Tinggi" on executing the function in university as the educational institute, research, and community service.
2. To actualized the mission of FMUI to become the best of 80 Medical University in the world in 2014
3. To give primary data for other researcher that need it.

1.5.3 Benefits for community

- The society will gain informationabout the effectiveness of Bti on solutions in controlling of DHF vector in water containers.
- The society will get information about which one of the water containers must be considered to prevent of *Ae.aegypti* mosquitoes breeding.

PART II

LITERATURE REVIEW

2.1. Dengue Hemorrhagic Fever

Dengue hemorrhagic fever (DHF) or known as *Demam Berdarah Dengue* (DBD) is an acute disease which characterized by four clinical symptoms, such as high fever, bleeding phenomena, hepatomegaly, and it is often accompanied by circulatory failure. In Indonesia DHF has become a major public health problem, because the prevalence and widespread distribution.[9]

Moreover, dengue is transmitted by the mosquito vector *Ae.aegypti*. Other potential vectors are *Ae.albopictus, Ae.polynesiensis*, and several other *Aedes* species. Dengue virus is transmitted from one person to another by mosquito saliva when the mosquito sucks the blood. The virus will be in blood circulation (viremia) for 4-7 days. A result of virus infection varies depending on a person's immunity that is asymptomatic, mild fever, *dengue fever* and *dengue haemorrhagic fever* (DHF/DBD). Patients who suffer asymptomatic and mild fever are an effective source of infection spreading.[9]

The important pathophysiology and determination of the extent of disease is that the enlargement of the plasma and homeostasis abnormalities that would manifest as an increase in hematocrite and thrombocytopenia. These types of abnormality are always in the course of the DHF disease. The existence of this plasma enlargement differentiates dengue fever and dengue hemorrhagic fever.[10] DHF is caused by dengue virus belonging to the genus Flavivirus which is family of Flavivirida. There are four serotypes of Den-1, Den-2, Den-3, and Den-4. Dengue virus that is an intracellular microorganism, needs replication of nucleic acid that could interfere with the process of host cells protein synthesis which can cause cell damage and death.

There are several clinical forms that are caused by dengue virus infection, for example febrile illness, viral syndrome and dengue fever (DD) or the heavy ones like dengue hemorrhagic fever (DHF) and dengue shock syndrome (SSD).

Clinical spectrum of this difference will result in a different prognosis with primary infection and also affect the prognosis. Almost 80% of secondary dengue virus infection are examined by hemaglutination inhibition test[11]

A treatment that has been done to eliminate dengue fever is to eradicate mosquito infecting by breaking the chain of transmission because the vaccine itself is still in the research stage and the virus drug procurement has not been found yet. [12-13]

2.1.1. Epidemiology

DHF disease made an initial transmission in 1968 through the port of Surabaya, while in 1980 was reportedly widespread in all provinces in Indonesia. The spread of dengue vector started from harbor to the village due to larvae that carried by some objects containing in the water in the transportation process. Indonesia is in a position of concern of DHF outbreaks in Southeast Asia, which is in the second below Thailand during the period 1985-2004.[14-15]

In the month of September to February DHF disease reportedly occurs widespread in Indonesia and reached a peak in December or January whereas in the big cities like Jakarta, Bandung, Yogyakarta, and Surabaya the transmission season occurs in March to August with a peak in June or July.[15]

There are some factors that are influences the increase and spread of DHF patients are very complex, because of population growth, unplanned and uncontrolled urbanization, the effectiveness in eradication of mosquito vectors in endemic areas, lastly the enhancement of transport vehicles. Morbidity and mortality of dengue is influenced by various factors such as immunological status of the host, vector density, viral malignancies, and local geographical conditions.[16]

DHF cases was increased in the year of 2004 and showed high numbers of cases known as an outbreak area (KLB) ,which are located in the Central Jakarta District (Tanah Abang and Kemayoran) , North Jakarta District (Koja and Tanjung Priok) West Jakarta District (Kebon Jeruk and Palmerah), South Jakarta

District (Pasar Minggu and Kebayoran Lama) and East Jakarta District (Ciracas and Kramat Jati).[17]

2.1.2. Area Demographics in Rawasari

Rawasari is village in the district of Central Jakarta bordering with East Cempaka Putih and West Cempaka Putih. Administratively this area is differentiates with three villages 30 of RW, 373 of RT, 18.556 Households, 79. 076 of people with population density is 16.872/km2.[7]

2.1.3. DHF Vectors

Ae.aegypti is an anthropophilic mosquito which came to a residential is the main carrier of dengue virus. This mosquitoe breeding in various water containers around the house. The larvae thrive as a basiceater ("bottom feeders") in clean water or dirty water-containing organic materials. The potential vector is *Ae.albopictus*. In Indonesia, particularly, the main vector is *Ae.aegypti* because of his living is in and around the house, while *Ae.albopictus* lives in gardens. *Ae.aegypti* are insects that are classified as :

-Phyla: Arthropoda

-Class: Insecta

-Order: Diptera

-Family: Culicidae

-Tribus: Culicini

-Genera: Aedes

-Species: *Ae.aegypti*

There are more than 24 synonyms of *Ae.aegypti* and some of them are, *Culex aegypti, Linnaeus, Culex fasciatus, and Fabricius*.[20-21]

2.2.Lifecycle of *Ae. Aegypti*

Ae.aegypti is an antropophilic and only female mosquitoes that bite. Female mosquito usually bites inside the house, but sometimes outside with less light like trees and areas of dense vegetation. At night the mosquitoes rest in houses in the hanging objects, such as clothing, bed nets, on the walls, and under the house near the place of breeding, usually in a dark place. In short period of time, this mosquitoes have a habit of multiple biting or called *multiple biters* that is biting several people because *Ae.aegypti* is a very sensitive and easily to get disturb. This situation is an advantage for *Ae.aegypti* in spreading dengue virus to multiple receivers at once so it was reported that some patients with dengue fever or DHF are in one house. Male mosquitoes are also attracted to humans when mating, but not bite.[18]

By the time the mosquito sucks human blood, in which the individual are happened to be suffering from dengue fever, dengue viruses has gotten into the mosquito body. The virus that is inhaled into the digestive tract, then entered into haemocoeloem and salivary glands. The virus takes 8-11 days to be able to breed well in propagative order to become infective (the bud sextrinsic). Therefore, the mosquitoes will remain infective for life. The virus is not found in mosquito eggs so that a conclusion can be made, there will be no *transovarian* (herediter) transmission.[18]

The frequency of mosquito bites or mosquito longevity is affected by seasonal changes. In the rainy season, DHF cases were more likely to increase. In Jakarta, a survey of *Ae.aegypti* biting showed that in the dry season mosquito bites are more frequent in the morning, while in rainy season high number of mosquito bites is on the day through the evening time. This shift allows *Ae.aegypti* vector to bite uninterruptedly while individuals sleep during the day time in the rainy season. Another possibility is that seasonal changes affect the virus or the man himself who changed his attitude to mosquito bites, such as using more time to stay indoors during the rainy season.[18]

After female mosquitoes rest their eggs on the container wall, the eggs will soon hatch into larvae within 1-2 days, the larvae will then turn into pupa in a span of 5-15 days. Pupa stage usually stands for 2 days. Under normal condition, the development of *Ae.aegypti* from egg to adult takes at least 9 days. Once it comes out of the pupa, mosquitoes will rest in the pupa skin for a while. That is when the mosquitoes wings are stretched and become stiff and strong so that they will be able to fly to suck blood. However, adult female mosquitoes have ready to suck human blood and in bred about a day or two days after leaving the pupa.[18]

Pupa of male *Ae. aegypti* hatches just before female. Male mosquitoes would not leave the nest as they are waiting for female to hatch and ready for copulation. After copulation *Ae.aegypti* will then suck human blood as much as he needed for eggs formation. The time it takes for the eggs to develop, from blood sucking until the egg is released, usually varies between 3-4 days. The time period is called gonotropic cycle. There are approximately 150 grains of eggs laid by female mosquitoes.[19]

Ae.aegypti usually lay their eggs on the afternoon before sunset. After laying eggs, female mosquitoes are ready to suck more blood. When the mosquito is impaired while sucking blood, the mosquito will bite the same person or others so that the virus will promptly transmitted to several people.

Generally, female mosquitoes will die in 10 days, but they have enough time for virus incubation (3-10 days) and virus spreading.[19]

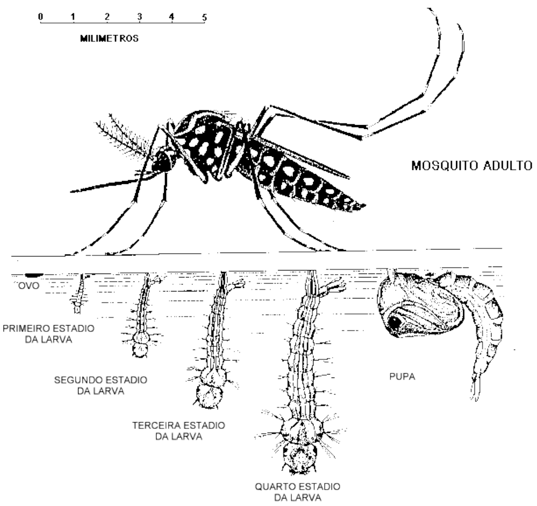


Figure 1 Mosquito Life Cycle *Ae.Aegypti*[22]

2.2.1. Identification of mosquito *Ae.aegypti*

a. Eggstage

The shape of *Ae.aegypti* eggs is oval like a torpedo with 0.6 mm length and 0.011 mg weight (Figure 2). By the time eggs are laid, the color is white, 15 minutes later it turns grey and after 40 minutes it becomes black. Under the compound microscope the surface of the egg appears like a wasp nest. Eggs are laid one by one on the wall of water containers (TPA) just about 1-2 cm above the water surface and the water is clear and is in the shade area with no direct sunlight. *Ae. aegypti* prefers stay in water-filled that is located inside the house or near the house than outside or distant from the house. The eggs can survive up to 6 months.[19]

Figure 2 Eggs of *Ae.aegypti*[24]

b. Larvae Stages

A larva of *Ae.aegypti* consists of head, thorax and abdomen. At the end of the abdomen there is an anal segment and siphon. In the figure 3 the larvae has characteristics of the open paddle at the anal segment, a pair of siphon feather, and pitchfork-shaped comb on the 7th abdomen. Larvae of *Ae.aegypti* is moving very swiftly and very sensitive to vibration and light stimuli. If there is a stimulus, the larvae will immediately dive for a few seconds then comes up to the surface of the water. The larvae takes food at the bottom of water container, they are the bottom feeder. By the time larvae inhales oxygen from the air, larvae places its siphon above the water surface so that the abdomen is seen hanging on the water surface.[19]

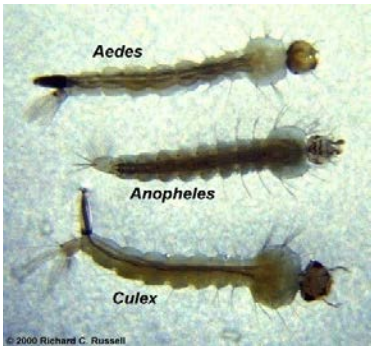


Figure 3 Larvae of *Ae.aegypti*, *Anopheles*, and *Culex*[23]

c. Pupa Stage

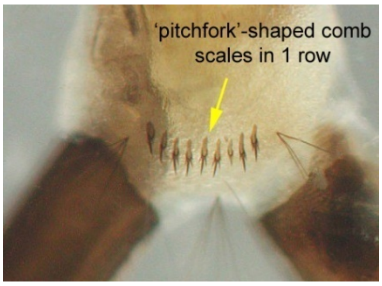
At this stage, in the figure 4 it shows that pupa has cephalothorax, abdominal and a paddle leg. Cephalothorax has a pair of triangular-shaped mouth piece to breathe. In distal abdomen, it has been found that a pair of paddle leg that is straight and narrow. Pupas are very active and tend to dive vigorously if disturbed before floating to the surface.[19]

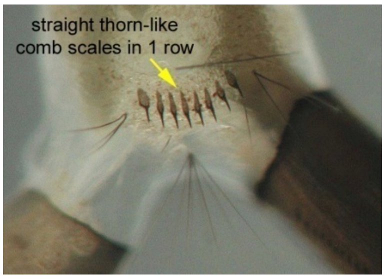
Figure 4 Pupa stages of *Ae.aegypti*[23]

d. Adult Stage

An adult mosquito body consists of a head, thorax and abdomen. Typical characteristics of *Ae.aegypti* that shows in figure 5 is the *lyre* in the thorax dorsal (mesonotum) that is a pair of parallel white lines in the middle and ticker crescent-shaped on the sides, black proboscis, white scaly scutellum, white abdomen on the basal, and white hind leg tarsus.[29]

Figure 5 *Ae.aegypti* and *Ae. Albopictus mosquito*[23]





2.2.2. Breeding ground

The eggs are placed in the inner wall of the water-filled container, slightly above the water surface[13]. *Ae.aegypti* breeding place is in the water containers (TPA) that contains clear water or a little contaminated water. *Ae.aegypti* prefers breeding place at the shaded area and cannot live in the area that has soil.[20-21]

The breeding places of *Ae.aegypti* can be grouped as follows:[24]

a. Water shelter (TPA) for every day purposes, such as: drums, tank reservoir, jars, bathtub /toilet bowl, and buckets
b. Water shelter (TPA) that is not for every day purposes such as canteens bird, flower vases, ant traps and used goods (tires, cans, bottles, plastic)
c. Natural water shelter (TPA) like: tree holes, rock holes, leaf midrib, coconut, banana bark, and bamboo strips

The existence of *Ae.aegypti* in a place in which a human needs to relocate water. In an area that has a water supply system, the population of *Ae.aegypti* is lower because households do not need to hold water in their containers. In contrast, in the area where there is no pipe water supply, the population of *Ae.aegypti* will likely to be high. Those areas that have salty water wells (sumur) with irregular drinking water supply for the households, people are tend to hold the falling water in the 200 liters water drum. There are lots of mosquitoes are breed inside it because of its size and the water is already there for quite some time.[19]

In fact, in an area with has a good water supply system, lots of people were still using the tub to relocate the water. That is because the habits of society, especially the Asian community who prefer to use water dipper, instead taking a bath with shower. Water in the jars and bath are always used, but usually not until they run out so that the larvae remain in place. Also when there is a movement, the larvae will move down so that it will not be wasted.[19]

The number of *Ae.aegypti* larvae breeding places affected by the rough-smooth walls of the water container, the color of the water container and water container capacity to absorb water. The number of larva eggs at the water containers with features like rough, dark and easy to absorb water are more high, so that the larvae are formed also increase. In contrast, the water container with smooth light colored and does not absorb water the number of larvae are fewer. A water container that is covered has more often containing larvae compared to place which is opened because in this place which in a darker room it is more preferred by female mosquitoes to stay.[19]

The numbers of larvae of *Ae.aegypti* are also influenced by the size of the water container and the amount of water contained in it. A large container with has more volume of water contains more larvae compared with water container consists of smaller amount of water. At the water container that contains water with a water level 2.5 cm, 5 cm and 7.5cm, eggs are laid about 60% on the container with the highest water surface.[19]

2.2.3. Factors Affecting the Development of Larvae

Larval development is mainly influenced by temperature and food. The average of larval development is 6-8 days. In the laboratory in optimal circumstances which is enough food and with water temperature of 25-27°C to make the larval development become longer in the respective temperatures, it should be set approximately higher than 28°C or less than 24°C. At 16°, 18°, 20°, 24°, and 31°C the development is respectively about 29, 24, 19, 10, and 12 days. Larvae died at temperatures less than 10° C or more than 40 degrees. At fluctuated temperatures the development will be faster than at constant temperatures.[25]

Larval diet should have contained essential nutrients such as proteins, lipids, carbohydrates, vitamin B complex and electrolytes. Foods that do not contain one of these essential substances will cause the death of larvae. In the larval food is natural microorganisms found in habitats such as algae, protozoa, bacteria, fungal spores and colloidal particles. Others microorganisms which are bacterial and fungal spores are the most important component for the larvae. Without bacterial and fungal spores, larvae cannot live even if other nutrients are available.[25]

Larval development is not much influenced by the pH of the water in the brood. In the wild, *Ae.aegypti* breeds in water with a pH of 5.8 to 8.6. At pH 3.6 to 4.2 or 9.2 to 9.5 percentage of adult mosquitoes that are formed only slightly reduced and larval development is only slightly more longer.[25]

2.2.4. Behavior of Adult Mosquitoes

Ae.aegypti mosquitoes sucking blood actively during the day with a second period of activities at 8:00 to 12:00 and 15:00 to 17:00. *Ae.aegypti* larvae prefers to suck blood in the house than outside the house and liked the place a bit dark. Female mosquitoes prefer the blood of humans rather than animals (nature anthropophilic). Unlike other mosquitoes, *Ae.aegypti* has a habit of sucking blood repeatedly until the stomach filled with blood (multiple bites) in a single cycle gonotropic, making it very effective as transmitters of disease.[25]

After sucking the blood, *Ae.aegypti* rests in the house or sometimes outside, close to breeding sites. Their common perch spot is hanging objects such as clothing, mosquitoes nets, or vegetation near breeding, where is usually in a rather dark and damp. In these places, mosquitoes are waiting for the egg maturation process. After resting and egg maturation process is complete, female mosquitoes will lay their eggs on the wall of breeding sites, just above the water surface. The eggs in a dry place can last for months at a temperature of -2 °-42°C, and if the place is filled with water the eggs can be hatched more quickly.[19]

Regular age of *Ae.aegypti* is usually around 10 days. The age is enough to replicate dengue virus in the mosquito's body. In the laboratory, with a room temperature of 28°C, 80% air humidity and the mosquitoes were fed 10% sugar solution and blood of mice, mosquitoes can reach the age of 2 months. Age of male mosquitoes is shorter than the female mosquitoes.[19]

2.2.5. Spreading

Ae.aegypti is widespread in tropical and sub tropic regions. Mosquitoes can live and thrive well into the height of ±1000m above sea level. Above the altitude of 1000m *Ae.aegypti* mosquitoes cannot breed because at that altitude the air temperature is too low so it is not possible for the life of the mosquito.[18]

Ae.aegypti is widespread throughout Indonesia, especially in port cities and in centers of dense population. The density of *Ae.aegypti* is shown highest in low-lying areas. That is probably because the population in low-lying areas more dense than the highlands.[14,19]

The ability of female mosquitoes fly an average of 40 meters, a maximum of 100 meters, but passively such as wind or carried by mosquito's vehicle can move any further. In some areas, clean water is placed in cans / tins and traded from house to house so that facilitate the spread of *Ae. aegypti*. The spread of the harbor to the village may be due to the larvae in a container carried through transportation.[14,19]

In the rainy season air humidity will increase. In addition, water containers are also greater because they filled by rain water, in this condition the populations of *Ae.aegypti* will increase. The one factor that led to increase of DHF transmission is the increasing of mosquito population.[19]

2.3. The Effect of Various Conditions in Water Containers by Density and Development of Larvae

2.3.1. Material influences The Density of *Ae.aegypti* Larvae in water containers

The number of larvae of *Ae.aegypti* in a container is made of ceramics at least compared with container made by *fiber-glass*, cement, drums and the number of larvae contained in the container are significantly different ($p < 0.05$). That means the number of larvae of *Ae.aegypti* is influenced by the type of container and container from ceramics can reduce the number of larvae of *Ae.aegypti* because it slicks and does not absorb air.[19]

Slippery walls of the container are the main factors which can affect the density of larvae. The wall of container which is made from rough wall is needed to attach the eggs and control the behavior of female mosquitoes lay their eggs. On the container walls are rough the mosquito can hold on tightly so that it can adjust the position of his body at the time when their egg is laid. Eggs are laid in the wall of the container on a regular basis 1-2 cm above the water surface. If the wall of container is smooth then the mosquitoes cannot be held tightly and cannot regulate his body so that the egg is fall on the surface of the water. Moreover, the eggs which attached on the slippery wall mostly fall on the surface. Eggs are diffused on the surface of the water mostly submerged and only 20% are hatched because the embryos die before the embryos are mature.[19]

The density of *Ae.aegypti larvae* in a container is influenced by other factors such as type, color and capacity of container to absorb water. The rough wall container can absorb water and dark light are the best places for *Ae.aegypti* to nesting. Instead, *Ae.aegypti* does not like the places on slippery walls, light and absorbed water of the container so it can prevent the oviposition. *Ae.aegypti* prefers to lay its eggs on the surface is rough and moist rather than dry and slippery surfaces. A study found that the number of eggs contained in the *ovitrap* which made by rubber is 1379 grains, 130 grains of plastic, 120 grains of cans and 30 grains of glass.[24]

For embryonic development in the egg takes a certain moisture content which obtained by inhibition at the container that does not absorb water so the inhibitions does not happen so that the embryos die of drought. Conversely, if the eggs submerged in water before embryos mature, then there is edema followed by the death of the embryo so that eggs cannot hatch. Ceramic is a material that does not absorb water so it can affect embryo development and it can be decreased the percentage of eggs that hatched. Thus the container is made of ceramics can reduce the density of *Ae.aegypti* larvae.[19]

2.3.2. Color Effect on Water Container (TPA) in the density of *Ae.aegypti* larvae

The density of *Ae.aegypti* larvae in a container is also influenced by the color. The container with dark color gives a sense of security and calm at the time that eggs are laid is much and the number of larvae that form is also more. In contrast to container with the light color the eggs are laid is much less. Based on a study reported that the number of eggs of *Ae.aegypti* was found in brown card board is 56%, 30% green card board, white card board 14%, 1% aluminum foil, and plastic transparent 0%. Besides the eggs of *Ae.aegypti* was found in a used car tires more than cans, bowls and other junk. That is because the tires are black and the surface is rough compared to other containers.[26]

The number of larvae of *Ae.aegypti* in a land fill are less brightly colored than the land fill dark and this difference was significant ($p<0.05$). That means the number of larvae of *Ae.aegypti* in a landfill is influenced by the colors and the color of light can reduce the number of larva. Based on the survey in some ceramics shops, color and fiber-glass ceramics are most likely in the public are dark colors like maroon and brown because it is not easy to look dirty. By knowing that the dark containers is contains more larvae than light colors which contain fewer larvae. Based on that society should be encouraged to use the container with light color. In addition, the light container, the larvae easily visible so that the larvae can be picked up by tools and the container does not need to be drained.[26]

2.3.3. Effect of TPA on the density of larvae type *Ae. aegypti*

The density of *Ae.aegypti* larvae in a container is also influenced by the type of containers. Based on the results of larval surveys have been carried out, it can be seen that type of container is found largely in the tub, drum and pail that is located inside the house. Breeding grounds are usually inside or near the house with condition of the water is relatively clean and often use for bathing or drinking.

Although the location is near the house or inside the house, as much as 89 % is used for bathing and washing and 92% of the water bath that comes from wells. Water in the jars in which comes from municipal water (PAM) by 74% and as much as 99% is used for drinking and cooking.[27]

2.3.4. The Effect of Density of Water Volume in Water Containers (TPA) against *Ae. aegypti* larvae

The density of *Ae.aegypti* larvae in a container is also influenced by the density of water volume in water containers. TPA which can accommodate more water is preferred by *Ae.aegypti* mosquitoes to lay their eggs. In addition to providing security and calm situations, the total volume of water can also effect of the water surfaces whether it is dark or not. Because, more volume the surface is darker while the containers with lots of volume which contained many of the foods is needed for the larvae to survive.

The average amount of water volume capacity is 173 liters in the house but in reality only 92 liters stored in a single charge. The total storage of water per house in the bath is two times more than jars and ten times more than the bucket. Therefore, many studies which state that more found of *Ae.aegypti* mosquitoes lay their eggs in containers that contain large volumes of water.[26] The long development of *Ae.aegypti* larvae in a variety of TPA is not significantly different ($p>0.05$), it means that the development of *Ae.aegypti* larvae is not influenced by the type and color of water containers.[28]

2.3.5. Location of Container influence on the density of larvae of *Ae.aegypti*

Hasyimi[26], reported that the *Aedes sp*. tend to laid their eggs in outdoors than indoors from house. The survey was conducted in Koja, North Jakarta, using mosquito egg traps (ovitrap) in water containers both inside and outside the house. The results shown after 8 months of the study revealed that the trap egg positive with eggs of *Aedes sp* and more commonly found in water containers outside than inside the house. From these studies it can be concluded that water containers outside the house is a comfortable places for *Aedes* to breed.

Trpis[28] revealed that there two types of containers for *Aedes sp,* for example artificial containers *(man-made breeding places)*, such as buckets, tin cans, bottles, drums, or jar, and natural container *(natural breeding places)*, such as holes in trees, coconut shell, the cochlea, or holes in the rock.

For *Aedes sp* to breed, Rosmanida[29] also revealed that there are significant differences between the density of *Aedes sp.* larvae outside the house than inside the house. The density of *Aedes sp* larvae, were higher in outdoors than indoors. Yotopranoto[27] reported that in Kaponan village, East Java, the *Ae.aegypti* larvae is commonly found more in container inside the house compared to the container outside the house. Hasyimi [26] also revealed that *Ae.aegypti* tend to have a place of rest and activity inside the house, while *Ae. albopictus* more live and breed in outdoors, such as in the bushes, gardens, and others.

2.4.Size of Population Density of *Ae.aegypti*

To determine the population density of mosquito larvae of *Ae.aegypti* in a location can be done by several surveys in selected houses.

2.5. Larvae Survey

Larval survey is important to do in all over the places or vessels that can become as breeding places of *Ae.aegypti* and to examine for the presence / absence of larvae. To check a larva in large water containers such as "kamar mandi", jars, drums and others, first thing is waiting approximately for a half until 1 minute to ensure the existence of larvae. After that, move a vase of flowers or bottles of water to check whether there is a larva or not. [30-31] The larvae surveys can be done with *single larval method* or visual. In the *single larval method,* a survey carried out by taking one larva in each container and put into a small bottle and identified. On the other hand, visual survey can be done enough to see whether or not larva each container without taking the larvae. In eradication programs of DHF commonly used larval surveys is a visual way. Size is used to determine the density of larval *Ae. aegypti*:[30-31]

a. *Wiggler-free numbers* (ABJ)

$$\frac{\text{Number of houses/buildings that are not found larvae}}{\text{Number of houses / buildings inspected}} \times 100\%$$

b. *House Index* (HI)

$$\frac{\text{Number of houses / buildings that are found larvae}}{\text{Number of houses/examined}} \times 100\%$$

c. *Container Index* (CI)

$$\frac{\text{The number of container containing larvae}}{\text{The number of containers inspected}} \times 100\%$$

d. *Breteau Index* (BI): The number of larvae in a container containing 100 houses / buildings

Water container means the place or vessel that can become mosquito breeding sites of *Ae.aegypti*. Free numbers of larvae and House Index further illustrate the extent of the spread of mosquitoes in an area while the Breteau Index shows the density and distribution of larvae.

2.6. Mosquitoes Survey

Mosquito survey is conducted by arrest mosquitoes bait by people inside and outside the house, each for 20 minutes per house and catching mosquitoes that alighted on the wall in the same house. Catching mosquitoes usually using an aspirator.[30-31]

Mosquito index used is:[30-31]

a. Bitting/landing rate

$$\frac{\text{The number of } \textit{Ae.aegypti} \text{ females caught by bait people}}{\text{The number of arrests x number of hours of arrest}}$$

b. Resting per household

$$\frac{\text{The number of } \textit{Ae.aegypti} \text{ female mosquitoes caught in mosquitoes perch}}{\text{The number of house arrest made}}$$

To find the average age of mosquitoes in an area, abdominal surgery mosquitoes captured to examine the state of her ovaries under a microscope. If the tip of the air pipe (tracheolus) the ovary is still rolling, it has never meant mosquitoes lay their eggs (nuliparous). If tracheolus already decomposed / roll apart, then it has been laying mosquitoes (parous). To know the age of mosquitoes is a newly hatched mosquito or mosquitoes that have used the old *parity rate*.[30-3]

Parity rate:

$$\frac{\text{The number of mosquito } \textit{Ae.aegypti} \text{ with parous ovaries}}{\text{The number of mosquitoes examined ovaries}} \times 100\%$$

When the results of a survey of an area entomologist, has a low rate parity means the mosquito population in the region mostly young, whereas when high rate parity indicates that the mosquito population in the region mostly old. The older the average age of the greater its potential as a mosquito vector. To calculate the average age of mosquitoes population in a more precise surgery of the ovaries of parous mosquitoes to calculate the amount of dilatation of the oviduct. Age of the mosquito population is the average dilatation gonotropic multiplied by one cycle.[30-31]

2.6.1.Terms of DHF Vectors

Not all *Ae.aegypti* can transmit dengue infection which necessary due to the vector of specified conditions. In the wild mosquito vector may be less than 5% because it does not qualify as a vector. The requirements to become a vector are as follows:

a. There is a source of infection for example DHF patients. Dengue virus present in the blood of patients1-2 days before the fever and is in the blood (viremia) patients for 4-7days.
b. Age mosquitoes is more than 10 days. The time required to ready infected virus requires 10 days to travel from the stomach to the mosquito salivary gland.
c. Mosquitoes to be resistant to the virus because the virus is also a parasite to mosquito.

2.7. Controlling of Dengue Hemorrhagic Fever[12]

2.7.1. Controlling of dengue vector

2.7.2. Before the transmission season

a. Individual Protection

Protection of *Ae.aegypti* bites can be done by eliminating mosquitoes breeding in the house by using mosquito nets in afternoon, setting a gauze on ventilation holes and apply mosquitoes eradicator. Moreover, spraying with is bought from drugs store also beneficial. DHF patients in the hospital also need to be given mosquito nets.

b. Mosquitoes Eradication Nest (Pemberantasan Sarang Nyamuk / PSN)

Mobilization of the PSN is a visit to the people house regularly at least every 3 months to do counseling and examination of larvae. The activity aims to counsel and motivate families and others to perform PSN, so that people house and public free from *Ae.aegypti* larvae / mosquitoes.

Before doing an activity in people houses, the first step is contacting of local leaders such as village heads, (RW and RT). After that held counseling to those leaders who continued without reach to the community. It is better, if done using a huge speaker, so everyone will hear the counseling. The next stage is collecting data, mapping the location, preparing the implementation of personnel, and preparing equipment. The aim of survey is to collect random data also to determine the average of the water containers, the volume of containers, types of containers and number of houses. Drawing map of the location as a direction to divide especially street /alley. The map is included also the location of the suspect cases/postal existing dengue laboratories. Each teams / officers who has been assigned in the survey area should be facilitated the implementation and supervision. The officers should receive training and practice in advance, among others, how to measure container, larvicide dose in water, and how to fill out the reports. Each officers are equipped with a bag/backpack, gloves, plastic/rubber, table spoon size of 10 grams, the meter length of ± 50cm, plastic bags, pencils and forms. PSN activities include clean the bath /toilet and other water containers at least once a week because of the development of egg-larva-pupa-mosquitoes is approximately 9 days, clean the inner wall of the tub regularly, and all the containers to get rid of mosquitoes eggs, close containers (jars, drums, etc.) so that mosquitoes cannot enter, clean the yard or the pages of cans, bottles, tires and shell so there is no mosquito breeding in these places, replace vase water and bird drink place to prevent drying on the roof or gutters, cover the holes with soil the tree or bamboo, dispose cans or bottles, and give health education to the community.

c. Mass Fumigation

There are 2 cycles of mass fumigation carried out from the house, especially in high endemic villages and public places such as schools, hospitals, health center.

d. Controlling of the Vector in the Countryside/ Village

The countryside or village that is pretended to be dangerous of DHF disease if in 3 years have been infected of DHF disease due to population density and transportation links between other areas. Controlling vector activities in area that is called as dangerous of DHF is carried out in accordance with the level of vulnerability of a region. There are four levels of vulnerability of DHF in countryside or village:

a. Vulnerability of countryside or village I (endemic)

Countryside or village in the last 3 years, have infected with DHF each year.

b. Vulnerability of countryside or village II (sporadic)

countryside or village in the last 3 years but not infected with DHF each year.

c. Vulnerability of countryside or village III (potential)

countryside or village in the last 3 years have never infected withDHF, but have population density, busy transport links with other regions, and have found larvae more than 5%.

d. **Vulnerability of countryside or village "free"**

countryside or village that never infected with DHF and height of the land more than 1000m above sea level, or a height less than 1000m but the percentage of larvae were found less than 5%.

2.8. Periodic examination of larvae {Pemeriksaan Jentik Berkala (PJB)

PJB is an examination in the water container and a breeding ground for *Ae.aegypti* mosquito larvae and to determine of existence, which is carried out in houses and public places on a regular basis at least every 3 months to observe the vector of larvae populations. This is done by visiting public houses to inspect the water container that is become breeding places of *Ae.aegypti* as well as providing education and information about PSN to the people. Regular, visit along with community outreach is expected that the society will be motivated to carry out the PSN in a regular basis. PJB in houses is made by a cadre or other larvae observers from the village. In the village which has labeled vulnerable I and II, each container that are consists of larvae is given by selective larvicides. PJB in public places is conducted by health workers, while the larvae which is found inside water container is treated with larvicide.

2.9. Larvicide

Larvicide is an application of Temephos (abate) to eradicate *Ae. aegypti* larvae. Temephos shaped is a granules that contained one million particles or 1% of temephos is enough for 10 liter of water. Larvicide on water containers have a residual effect for 2- 3months. In a year, when some areas using larvacide by the people, population of *Ae.aegypti* will be controlled and can be suppressed. Larvicide application was firstly made two months before the high transmission season in a certain area or in areas that had never been infected with dengue. Therefore, the application performed 2 - 2.5 months later (at the time of transmission / *Ae.aegypti* populations are highest). Applications can be made 2-2.5 months after the second application.

2.9.1. Prevention Focus

Counter measures include the focus of epidemiological research activities, group counseling and fumigation. Epidemiological studies are done by the larvae inspection in infected houses and people (who were treated in hospital / health center) and other nearby houses. Patients who are students will also carried out the larvae examination in school and the houses around the school if necessary.

Group counseling are given to the citizens of dwelling patients by health center staff or volunteers. Counseling to all students at the school is done by the teachers. In this counseling, examination results of the larvae is reported and the people are asked to do the PSN.

Fumigation is carried out if:

a. House Index at residential premises with ≥ 10% or found more than 1 patient in the administrative region within a month, carried out fogging in all the administrative region.
b. Administrative units in a region where there are 2 people or more in a distance less than 4 weeks / 1 month.
c. In an administrative area within one week of an increase in the number of patients twice or more compared with the previous week, conducted fogging in all areas contained regional patient in the previous week or last 2 weeks.
d. In an administrative region within a month there is an increasing number of patients with twice or more compared with the previous month or compared with the same month the previous year, is conducted fogging

method in areas of existing patient in the last month and the following month.

e. At the school where people found *Ae. aegypti* in some places, carried out fogging at school and houses (includes houses around the school). Fumigation is carried out with minimum two times within ten days at patients house and their surroundings with a distance of 100 meters around the house of patients.

2.9.2. Counter measures outbreak / epidemic

Counter measures outbreaks / epidemics of mass fumigation carried out by two cycles, and mass mobilization larvicide PSN throughout the affected region. Mobilizing communities to PSN also be implemented in the region / surrounding area or have a risk of spread of an outbreak of plague.

2.1.1. *Bacillus thuringiensis*

Currently it was recently found a number of agents that are proven to be effective to eradicate mosquitoes. One of them is *Bacillus thuringiensis*, especially serotype H-14:29. *Bacillus thuringiensis* bacteria is a gram-positive bacillus that produces oval-shaped spores. Nature is facultative anaerobes. During the period of sporulation, this bacterium produces a crystal protein (cuboidal, triangle, and diamond-shaped) crystals called endotoxic. These crystals high specificity against mosquito larvae of the genus *Culex, Anopheles, Aedes, Mansonia, Psorophora, and Simulids*.[27]

2.1.2. Genetic Characteristics of *Bacillus thuringiensis*

a. Optimal temperature for growth is 28-30° C

b. Optimal pH: 7.2-7.4

c. Sunlight exposure for long periods of time may reduce the stability and effectiveness are dependent on the amount of ultraviolet radiation.

d.Desiccation

Each desiccation can cause the protein crystals become inactive e.Characteristics

Serotype H-14 has all the biochemical and morphological characteristics common to all types of Bti. The only difference can be found in the vegetative cell flagellar antigen, the antigen composition of delta endotoxin, in the type of esterase. In this type there is no beta-exotoxin available, which remained stable to heat and can produce protein crystals in the form of the protein that possible. This is what differentiates with other variants of Bti, which generally produces a diamond-shaped crystals .[27]

2.1.3. Physiology of *Bacillus thuringiensis*

In infancy, *Bacillus sp*. has three phases, namely the vegetative phase (growth and division of microorganisms), sporulation phase (there is a change in the cytoplasm, endonuclear proteolysis and cell rearrangements), and the late sporulation phase where there are crystals of protein synthesis by larval activity.

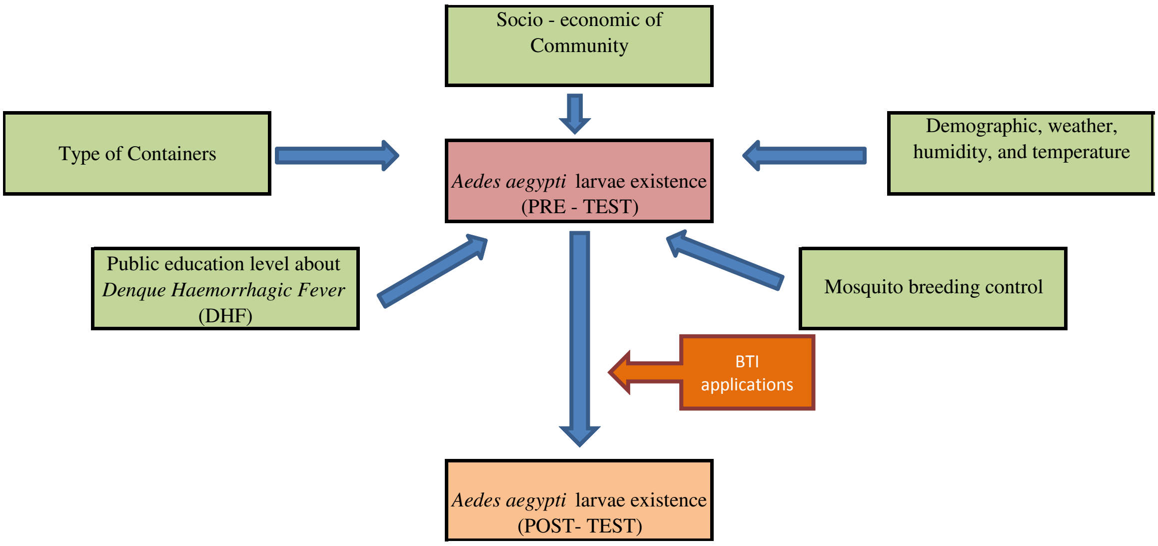
2.1.4. Mechanism of Action

Mosquito larvae can ingests pores and protein crystals. The toxic fragments that are released will be recognized by specific receptors located on intestinal epithelial cells. This process will generate holes in the membrane of the distal micro capillarity in cecum mucosa and the central part of the intestine. Some changes that happen in organelles in the cytoplasm would occur simultaneously, which includes the disintegration of the cytoplasm, mitochondria tumefaction and dilatation of perinuclear space. As a result it will trigger the collapse of tissue hypertrophy of intestinal cells in the small intestine and caecum colon and will lead to ionic imbalance, toxemia and bacteremia which leads to death of larvae.

2.1.5 Protection measure

Bio larvicide does not result in operational risk. Concentration of active ingredients and raw materials also contain no toxic effects to humans and animals. Its products are not considered harmful and specifically on mosquito larvae

2.2 Conceptual Framework



PART III

METHODS

3.1. Research design

This study is using a *quasi-experimental* design with intervention of Bti applications.

3.2. Time and place of research

The data collection was carried out only in one village which is Rawasari Village. This data was done by 2 periods of time, started from 14th February 2010 and 15th March 2010. However, the process of the study has been taken approximately for 3 years which was accounted since this study started a month before the first surveyed until 8th July 2013 in the final research presentation. The identification of larval was held in Parasitology Departement and the process was finished up to 2 weeks. Furthermore, the data was analyzed statistically by using SPSS 20 version for 3 months.

3.3. Population observed

3.3.1.Population target

The target population in this study was all containers which was located in Rawasari Village.

3.3.2. Population Range

In this research, the population range was all containers that filled with water which is located in Rawasari Village on 14th February 2010 and 15th March 2010

3.4 Research Subject

The research subject was all containers with or without *Ae.aegypti* larvae which located in or around 120 houses in Rawasari Village on14th February 2010 and 15th March 2010.

3.5. Random sampling and methods of sampling

The survey conducted in 100 houses randomly of selected houses in one village. Then, all containers were sampled that fulfill the criteria of inclusion and exclusion. Moreover,This survey carried out by single-larval methods in Outdoor and indoor containers where each containers were taken only one larva by using a water dipper. After that, the larva was identified using a microscope in laboratory of Parasitology FKUI. In addition, as an object to avoid of the drop-out, the data added up to 20 houses.

3.6. Inclusion and Excluson Criteria

3.6.1 Inclusion Criteria:

- The entire of indoor and outdoor containers that filled with water and around houses with or without larvae.

3.6.2 Exclusion Criteria:

- The containers where the location was not affordable with the researchers.

3.7 Variable Identification

3.7.1 Independent variable : The application of *Bacillus thuringiensis israearlensis*

3.7.2 Dependent variable : The water containers

3.8. Working Procedure

3.8.1 Materials and Tools

1. Alcohol 70%
2. Hot Water
3. Microscope
4. Labeling paper
5. Survey Forms
6. Glass tube and covering
7. Small pipette
8. Dipper
9. Small bottle

10. Plastic glass
11. Filter paper
12. Microscope
13. Pencil and notebook
14. Flashlight

3.8.2. Data Collection Method

At the first visit, the survey was conducted by observing all larvae from containers inside and outside of 100 houses. The number of houses selected by simple ramdom sampling method to choose 100 houses, also added 20 houses to avoid *drop-out.* Using flashlight to illuminate th containers with the intention to make it easier when looking for larvae. If, there are larvae in the containers using a scoop with elevation 45 degrees towards the larvae clusters. Afterwards, the larvae were taken using a small pippette, then transfer into the small bottle and given information with a label. After that, the containers will be given $4ml/m^2$ of Bti concentration.

All containers which containing of larvae or not will be recorded in the forms. Furthermore, the larvae will be obtained and identified by using a microscope, also identification of key species. Thus, the results will be tabulated and the density index of larvae will be counted. One month later, student had to do re-entomologi survey (*posttest)* to evaluate the results of Bti applications.

3.9. Management Planning and Data Analysis

The data from the survey forms are grouped based on variables. The Data is tabulated by region and the presence of larvae in the containers before and after application of Bti. After that, all the data tabulation is done by using microsoft excel 2007. Morover, to test the presence of *Ae.aegypti* larvae before and after application of Bti in Rawasari,the researchers have to use McNemar test statistics by using SPSS for windows version 20. Lastly, making the conclusion from the analysis result and adding some suggestion to evaluate people in Rawasari Village about the importance of preventing DHF disease.

3.10. Operational Definition

3.10.1 Container is a place to relocate water, either natural or made by human that could be a breeding place for *Ae.aegypti* larvae.

3.10.2 Indoor *container* is the container that is located inside the house.

3.10.3. Outdoor *container* is the container that is located outside the house.

3.10.4 Water *container* (TPA) is a container that can relocate water for 5 litres

3.10.5 Non-water *container* (Non-TPA) is a container that only fill in with water less than 5 litres

3.11. Research Ethics

This research will involve animals, i.e. the vector *Ae.aegypti*. In order for identifying the type of larvae, the larvae will be killed first by pouring high temperature water inside the larvae container. The researcher will done the research in civilliant's house and the researchers have to show good behavour and introducing their self first. In order to minimilize discomfortness of the house owner due to stranger, the researchers will be accompanied by "jumantik" from Rawasari region with coordination with local governor. Before doing the survey,the students asked a permission to the landlord and explain the purpose of the study. If the landlord agrees, then the researchers will observe the container that was in the house and drop the Bti solutions into the containers. The researchers will maintain the confidentiality of the survey results. Once the survey is completed, the researchers will give some souvenirs to the owner of the houses as a sign of gratitude. If the owner's house does not agree, then the researchers will look for another house.

PART IV
RESULT

Table 4.1 The existence of *Ae.aegypti* larva inside water containers (TPA) before and after application of Bti in Rawasari Village

Type of containers	Before		After	
	+	-	+	-
“Bak mandi”	7	73	5	75
Toilet Tub	2	0	1	1
Drum	1	10	1	10
Crock	0	8	0	8
Bucket	4	90	5	89
Others	1	7	0	8
Total	15	188	12	191

In the table 4.1 it shows that before Bti applications, the total number of water containers which were containing of positive larvae was 15 from 100 houses that have been identified. These water containers were “Bak mandi”, toilet tubs, drums, buckets, and other type of water containers. The highest number of water containers that contained *Ae.aegypti* larvae was “Bak mandi” which accounted for 7 positive larvae. Howerer, Drum was stated as the highest number of TPA which contained larvae in proportion up to 9,1% (1 positive from 11, compared with “Bak mandi” that only 8,75% (7 positive from 80)

After Bti applications, the number of positive larvae inside water containers decreased slightly to 12 from 203 of water containers that have identified in 100 houses. There were 4 types of water containers that contained of larvae, for example “Bak mandi”, toilet tubs, drums, and buckets. “Bak mandi” and buckets had the same number of positive larvae that was 5 larvae. But, toilet tub indicated as the highest number of positive larvae in proportion according to types of water containers, which reported for 50% (positive 1 from 2, compared with “bak mandi” that only 6,25% ;positive 5 from 80, and buckets just about 5,32% ; 5 positive from 94)

After application of Bti, “bak mandi” still remains as the most water containers that contained larvae, but the positive of larvae decreased from seven to five. On the other hand, there was a decrease of positive larvae in toilet tub and drum, while in other water containers there was an increase in the number of positive larvae.

Table 4.2 The existence of *Ae.aegypti* larvae in water containers before and after Bti applications in Rawasari Village

Water containers	Positive	Negative	Significant test
Before	15	203	*McNemar*
After	12	203	p = 0,629

It can be seen that there was a slight fell in the number of positive larvae inside water containers in Rawasari Village after Bti applications. This difference is not significant after testing with McNemar test that showed p= 0,629.

PART V
DISCUSSION

From entomology survey, it was found that after applications of Bti in Rawasari village, Bti cannot reduce the presence of larvae in water containers and in McNemar test the differences between before and after Bti applications was not significant. The result is different with Fansiri et al[32] statements that Bti tablets can kill *Ae.aegypti* larvae in the *tempayan container* which is containing 200 liters of water and can kill the larvae up to 11 weeks. Benjamin et al[22] reported about the results of his research in Malaysia is that the persistence of Bti in granular form can survive for 25 days, while in Salatiga, Boewono et al[33] stated that the efficacy of Bti tablets can survive for seven weeks. Bti efficacy is high in all three of these studies due to the use of Bti tablets / granules that sink to the basis of water containers and they are known as *slow-release.*

The use of slow-release formulation will make the toxin that contained in the preparation of Bti is released slowly into the water. After that, when the water is used and then added back, there is no dilution occurs because the toxin particles continuously released, so that the concentration of Bti is not reduced and remains effective for killing the larvae. In this study, the type of Bti is a solution that soon dissolved in the water when it dropped, therefore if the water is used, then the Bti is dissolved in the water and it will be wasted. If the containers filled with water again, then the dilution of Bti will occur, while the effectiveness of Bti is really influenced by concentration in the water (LC95). LC95 Bti is required to kill the *Ae.aegypti* larvae which was accounted for 10 ng / mL or 1 mg/m3 and containing approximately 103 cells of bacteria.[32]

Another factor that influences the effectiveness of Bti is type of water containers. The surface of water container that is rough and porous will make easier the attachment of the toxin on the floor / wall of water containers. In contrast, the toxin cannot be attached to the water containers with a smooth surface, so that the persistence of the toxin is not too long due to waste with water. Generally, people in Rawasari Village are used water container which are made of plastic, fiber, and ceramics with a smooth surface. Benjamin, et al[22] reported that the Bti toxin is more effective in containers which is made of clay (pitcher) than plastic and ceramic. This is because, these materials do not have pores like clay that can be used as a place of attachment of Bti toxins. The effectiveness of Bti in this study is due type of Bti which is known as a solution and type of containers that are used in the community.

Generally, *Bak mandi* and *Ember* were the most water containers that have found larvae positive, because the size are large and can fill in lots of water. In addition, these water containers are found in almost every houses, because people prefer to use *gayung* (bucket bath) than *shower.* Sungkar[34] reported that people in Southeast Asia are tend to use a *gayung* (shower bucket) than *shower,* thus they need to relocate the water first before they are getting shower. To conclude, I think Bti cannot decrease the presence of *Ae.aegypti* larvae because the concentration of Bti could decrease because of dilution. The important things is lacking of awareness of people in Rawasari Village about the importance to protect them from DHF disease.

BAB VI
CONCLUSION AND SUGGESTION

6.1 CONCLUSION

To conclude, before application of Bti the number of larvae in the water containers was high which accounted for 15 larvae, while after application of Bti the number of larva decreased slightly to 12. Thus, it means that Bti application cannot decrease the number of larvae in water containers in Rawasari Village. Moreover, Drum was stated as the highest number of TPA which contained larvae in proportion up to 9,1% (1 positive from 11, compared with “Bak mandi” that only 8,75% (7 positive from 80). These types of water containers were mostly found of *Ae.aegypti* larvae.

6.2 SUGGESTION

It needs further examination to enhance the effectiveness of Bti liquid and needs to be replaced with a *slow-release* tablet form.

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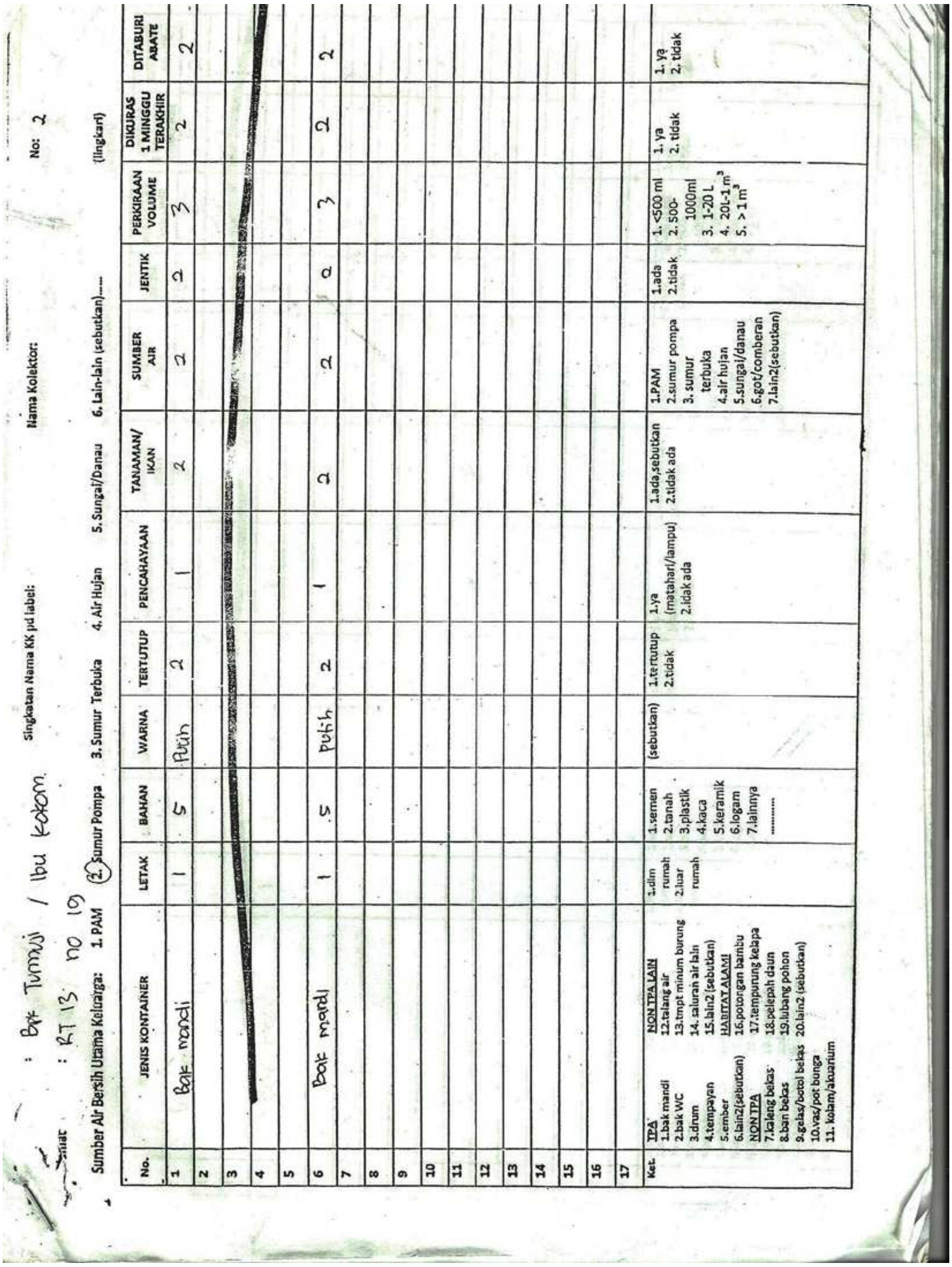
APPENDIX

Appendix1.Survey Questionnaire.



: Bpk Turmuji / Ibu Kokom. Singkatan Nama KK pd label: Nama Kolektor: No: 2

amat : RT 13 no 19

Sumber Air Bersih Utama Keluarga: 1. PAM 2. Sumur Pompa 3. Sumur Terbuka 4. Air Hujan 5. Sungai/Danau 6. Lain-lain (sebutkan)...... (lingkari)

No.	JENIS KONTAINER	LETAK	BAHAN	WARNA	TERTUTUP	PENCAHAYAAN	TANAMAN/ IKAN	SUMBER AIR	JENTIK	PERKIRAAN VOLUME	DIKURAS 1 MINGGU TERAKHIR	DITABURI ABATE
1	Bak mandi	1	5	Putih	2	1	2	2	2	3	2	2
2												
3												
4												
5												
6	Bak mandi	1	5	putih	2	1	2	2	2	3	2	2
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
Ket.	TPA 1.bak mandi 2.bak WC 3.drum 4.tempayan 5.ember 6.lain2(sebutkan) NON TPA 7.kaleng bekas 8.ban bekas 9.gelas/botol bekas 10.vas/pot bunga 11. kolam/akuarium NON TPA LAIN 12.talang air 13.tmpt minum burung 14. saluran air lain 15.lain2 (sebutkan) HABITAT ALAMI 16.potongan bambu 17.tempurung kelapa 18.pelepah daun 19.lubang pohon 20.lain2 (sebutkan)	1.dlm rumah 2.luar rumah	1.semen 2.tanah 3.plastik 4.kaca 5.keramik 6.logam 7.lainnya	(sebutkan)	1.tertutup 2.tidak	1.ya (matahari/lampu) 2.idak ada	1.ada,sebutkan 2.tidak ada	1.PAM 2.sumur pompa 3. sumur terbuka 4.air hujan 5.sungai/danau 6.got/comberan 7.lain2(sebutkan)	1.ada 2.tidak	1. <500 ml 2. 500-1000ml 3. 1-20 L 4. 20L-1 m^3 5. > 1 m^3	1. ya 2. tidak	1. ya 2. tidak